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Effect of ginsenoside Rb1 on oxidative damage of human retinal pigment epithelial cells induced by 4-hydroxynonenal

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Abstract. Objective: To study the protective effect of ginsenoside Rb1 on oxidative damage of human retinal pigment epithelial cells (ARPE-19) induced by 4-hydroxynonenal (4-HNE) and its mechanism. Methods: CCK-8 method was used for detecting the concentration of ginsenoside Rb1 and 4-HNE. ARPE-19 cells were randomly divided into Control Group, Rb1 Treatment Group, 4-HNE Group and 4-HNE+RE1 Treatment Group. The morphological structure of cells in each group was observed by light microscope, and the content of reactive oxygen species (ROS) in cells was detected by fluorescence microscope. Western blot was used for detecting the expression level of apoptosis related factors B cell lymphoma (Bcl) - 2 and Bax protein. Results: CCK-8 showed that the concentration of ginsenoside Rb1 was 100 μmol/L when ARPE-19 cells have the highest proliferation rate and better protection, so 100 μmol/L ginsenoside Rb1 for subsequent experiments. Compared with 4-HNE Group, 50.0μmol/L and 100.0 μmol/L. The cell activity in ginsenoside Rb1 Group was significantly increased ($P<0.05$). Compared with the Control Group, the ROS content in cells of 4-HNE Group increased significantly ($P<0.05$), the expression of Bax protein increased, and the expression of Bcl-2 protein decreased. Compared with 4-HNE Group, 4-HNE+Rb1 Treatment Group significantly reduced the intracellular ROS content ($P<0.05$), Bax protein expression decreased, and Bcl-2 protein expression increased. Conclusion: Ginsenoside Rb1 can improve the oxidative damage of RPE cells induced by 4-HNE, and the mechanism may be related to the reduction of apoptosis by ginsenoside Rb1.

Keywords. Ginsenoside Rb1, 4-hydroxynonenal, Oxidative damage, Human retinal pigment epithelial cells.

Age related macular degeneration (AMD) is a senile degenerative ophthalmic disease [1]. Its pathogenesis is still unclear, but oxidative stress reaction acts on retinal pigment epithelium (RPE), causing mitochondrial damage and reducing the production of reactive oxygen species (ROS) [2,3]. With the gradual growth of age, the activity of peroxidase in the human body decreases, and retinal RPE suffers from oxidative damage, leading to AMD, which can seriously cause vision loss and affect the quality of daily life [4]. Therefore, it is an effective way to protect RPE cells from AMD by playing an antioxidant role. 4-hydroxynonenal (4-HNE) is an aldehyde compound, which exists in cell mitochondria and is an important substance leading to mitochondrial dysfunction [5]. Oxidative stress reaction can cause the accumulation of 4-HNE, destroy signal pathways, induce inflammatory reaction and generate apoptosis signals, and participate in various cellular toxic reactions, causing the occurrence and development of oxidative stress related diseases [6,7]. Ginsenoside Rb1 (Rb1) not only has a protective effect on cardiovascular system, central nervous system, immune system and other systems [8], but also has a variety of effects, such as promoting bone formation, enhancing immunity, resisting skin aging, and eliminating oxygen free radicals [9]. At present, whether ginsenoside Rb1 can protect RPE cells from oxidative damage and the related mechanisms are still unclear. In this study, 4-HNE was used for damaging RPE cells to establish an oxidative damage model in vitro, and the protective effect and mechanism of ginsenoside Rb1 on RPE cells were analyzed.

1. Materials and Methods

1.1. Materials

Human retinal pigment epithelial cell ARPE-19 (American ATCC Company); Ginsenoside Rb1 (Chengdu Purifa); 4-HNE, dichlorodihydrofluorescein (DCFH-DA, Sigma, USA); Fetal bovine serum (Gibco Company, USA); DMEM/F12 medium, fetal bovine serum (HyClone Company, USA); CCK-8 Cell Activity Test Kit (Dojindo Company, Japan); B-cell lymphoma (Bcl) - 2, Bax β- Actin antibody (American CST Company); CO_2 incubator (Thermo Company, USA); Fluorescence inverted microscope (Japan Olympus Company); Enzyme microplate analyzer (BIOTEK Company, USA).

1.2. Cell culture

Human ARPE-19 cells were cultured in DMEM/F12 medium containing 10% fetal bovine serum, 1% culture of 100mg/L penicillin and 100mg/L streptomycin, and cultured in 5% CO_2 incubator at 37 ℃. When the cells grow and fuse to 80%, the culture medium is changed regularly and the cells are propagated. Cells in logarithmic growth period are selected for subsequent experiments.

1.3. CCK-8 assay for cell viability

After subculture, ARPE-19 cells contain 5×10^3 cells were inoculated in 96 well cell culture plate as standard, and 200μL cells were inoculated in each well cell suspension is inoculated into the cell culture dish with culture medium, and the culture dish is replaced twice after 1w. The cells were replaced with serum-free culture medium after adherent propagation. Ginsenoside Rb1 solutions of different concentrations were added to the culture dish for 24h. The

concentration of Ginsenoside Rb1 was 0.0, 12.5, 25.0, 50.0, and 100.0μmol/L respectively, 10 μl CCK-8 solution was added to each hole after 24h, the culture plate was placed in the incubator for 3h, the cells under the microscope were observed and collected. The experiment was repeated for three times. The survival rate of ARPE-19 cells was detected with CCK-8 kit. The wavelength of 450nm was selected. The absorbance (OD) of each hole was measured with the microplate reader, and the cell proliferation rate was calculated.

1.4. Cell grouping and administration

10 μmol/L 4-HNE was selected as the appropriate concentration for administration [10], and then the optimal concentration of drugs was selected according to CCK-8 method, and 100 μmol/L ginsenoside Rb1 was used as the optimal concentration. Then, ARPE-19 cells were divided into Control Group, Rb1 Treatment Group, 4-HNE Group and 4-HNE+Rb1 Treatment Group. Control Group: routine culture; Rb1 processing Group: 100 μmol/L Rb1 was added for 24h; 4-HNE Group: 10 μmol/L4-HNE cells were added for 12h; 4-HNE+Rb1 Treatment Group: 100 μmol/L Rb1 for 24h, then 10 μmol/L 4-HNE was added for 12h. The arrangement, size and morphology of ARPE-19 cells in each Group were observed under the light microscope.

1.5. Determination of ROS content in cells by fluorescence microscope

The cells after grouping treatment were inoculated into a 24-hole fluorescent enzyme label plate with a diameter of 14 mm for climbing. After good adhesion, the cells were placed in the incubator and cultured for 24h to promote cell adhesion growth. The cells were rinsed with phosphate buffer solution (PBS) for 1min for three times. The fluorescent dye DCFH-DA was diluted with serum free culture medium at a concentration of 10 μmol/L. It was placed in a cell incubator with a temperature of 4 ℃ and incubated in a dark place for 10 min. After incubation, it was washed again with PBS for three times. The ROS content in cells was detected by fluorescence labeling under a fluorescence microscope.

1.6. Western blot detection of protein expression level

Cells of each group were collected and 50 μL Protein lysate (including PMSF) was added for splitting on ice for 30min, centrifuged at 4 ℃ for 15min, the total protein of cells was extracted, the protein was quantitated with the method of biquinoline formic acid (BCA), and 50 μg protein sample was taken for electrophoresis on 10%~15% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel, and the protein was transferred to polyvinylidene fluoride (PVDF) membrane. After 5% skimmed milk powder was sealed for 2h, Bax, Bcl-2, and β- actin antibody was incubated overnight at 4 ℃. On the next day, TBST was washed for three times, horseradish peroxidase (HRP) labeled goat anti-rabbit IgG was added, and incubated at room temperature for 1h. After TBST was washed for three times, ECL chemiluminescence developer was added, and the relative protein expression of Bax and Bcl-2 was calculated with Image J gel image analysis system.

1.7 Statistical analysis

SPSS21.0 software was used for one-way ANOVA and LSD-*t* test.

2. Results

2.1. Effect of ginsenoside Rb1 on ARPE-19 cell viability

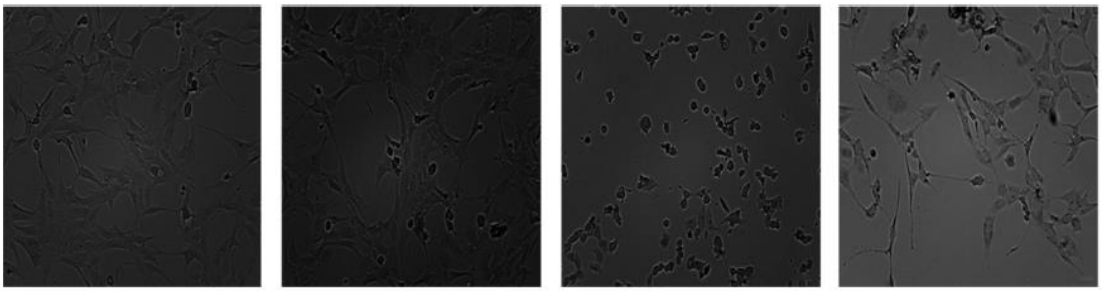
0.0, 12.5, 25.0, 50.0, 100.0 μmol/L Rb1 was acted on ARPE-19 cell for 24 respectively, which proliferation rate was (100.00 ± 0.37) %, (101.30 ± 0.64) %, (101.36 ± 0.28) %, (102.59 ± 0.85) %, and (103.21 ± 0.57) %, respectively. There was a statistically significant difference in cell viability among the Groups ($P<0.01$). After treatment with 50.0 and 100.0 μmol/L ginsenoside Rb1, ARPE-19 cell activity increased significantly ($P<0.05$).

2.2. Effect of ginsenoside Rb1 on the activity of ARPE-19 cells induced by 4-HNE

The cell activity of the Control Group, 4-HNE Group, 4-HNE+50 μmol/L Rb1 Treatment Group, 4-HNE+100 μmol/L Rb1 Treatment Group was (100.00 ± 0.72) %, (66.39 ± 1.14) %, (84.02 ± 0.96) %, (89.41 ± 1.11) %, respectively, and there was significant difference between the four groups ($P<0.001$). Compared with the Control Group, 4-HNE Group was significantly lower ($P<0.05$); 50 and 100 μmol/L Rb1 Treatment Group was significantly higher than 4-HNE Group ($P<0.05$), and the change in 100 μmol/L Rb1 Treatment Group was more significant ($P<0.05$). In this study, the action concentration of ginsenoside Rb1 in subsequent experiments was selected as 100 μmol/L.

2.3. Effect of ginsenoside Rb1 on morphology of ARPE-19 cells induced by 4-HNE

In the Control Group, the cells were spindle shaped and conventional adherent; in 4-HNE Group, the cells were shrunken, less in number, poorly shaped and abnormally arranged, and some cells could not adhere to the wall; the cells in 4-HNE+Rb1 Treatment Group were gradually relieved and their morphology was improved. The cells in Rb1 Treatment Group were similar to those in Control Group, with no significant difference, as shown in Figure 1.

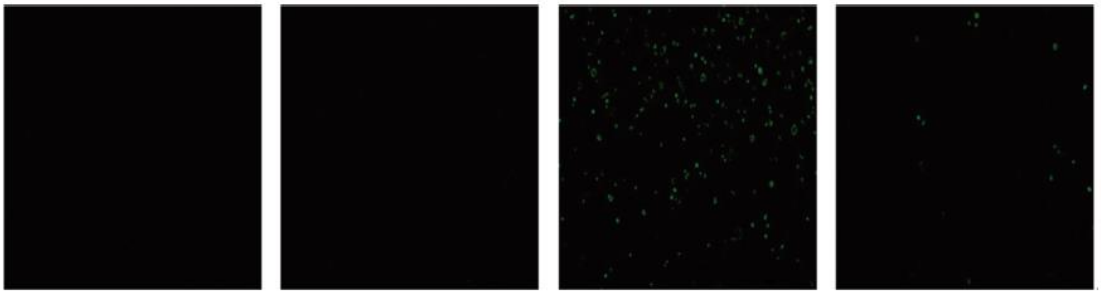


Control Group | Rb1 processing Group | 4-HNE Group | 4-HNE+Rb1 Treatment Group

Figure 1. Effect of ginsenoside Rb1 on morphology of ARPE-19 cells induced by 4-HNE （ × 40)

2.4. Observation of the effect of Rb1 on ROS production of cells in each Group under fluorescence microscope

The Control Group and Rb1 Treatment Group showed almost no green high fluorescence, while the 4-HNE Group showed enhanced green high fluorescence signal compared with the Control Group; Compared with the 4-HNE Group, the 4-HNE+Rb1 Treatment Group showed a weakened green high fluorescence signal, as shown in Figure 2.



Control Group | Rb1 processing Group | 4-HNE Group | 4-HNE+Rb1 Treatment Group

Figure 2. Effect of ginsenoside Rb1 on ROS production in ARPE-19 cells induced by 4HNE (× 40)

2.5. Effect of ginsenoside Rb1 on expression of Bax and Bcl-2 proteins in ARPE-19 cells induced by 4-HNE

The expression of Bcl-2/Bax in the Control Group, 4-HNE Group and 4-HNE+Rb1 Treatment Group (1.85 ± 0.16, 0.36 ± 0.05, 1.03 ± 0.06) was significantly different ($P<0.001$). Compared with the Control Group, the expression of Bcl-2/Bax in 4-HNE Group decreased significantly ($P<0.05$); Compared with 4-HNE Group, 4-HNE+Rb1 Treatment Group was significantly up-regulated ($P<0.05$), as shown in Figure 3.

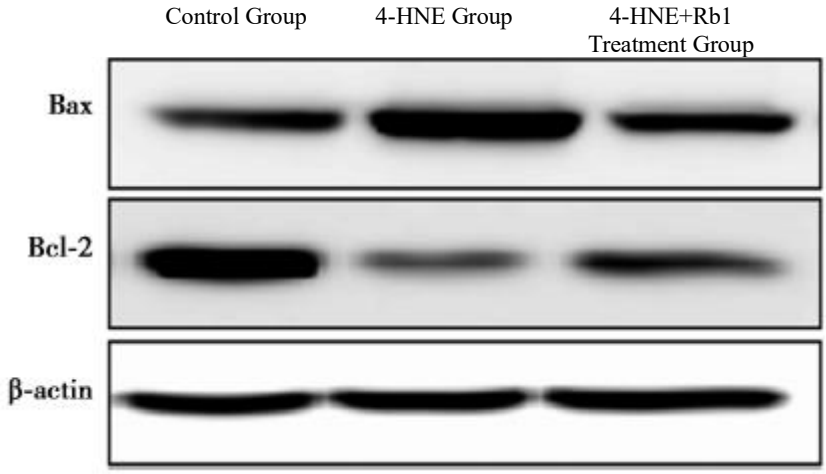


Figure 3. Effect of ginsenoside Rb1 on the expression of apoptosis protein in ARPE-19 cells induced by 4-HNE

3. Discussion

With the accelerated aging of the daily population and the gradual prolongation of life expectancy, AMD has become one of the reasons for the decline of vision in the elderly. At present, most AMD patients are treated with anti-vascular

endothelial growth factor (VEGF) in clinical practice [11]. Some studies have shown that oxidative stress is one of the causes of AMD [12], and with the gradual growth of human age, there are more and more oxidative stress reactions in the body, resulting in the gradual aging of various organs, further aggravating the damage to the retina [13], causing damage to retinal photoreceptors. The oxidative stress reaction of RPE layer cells as a pathogenic factor of AMD has been confirmed [14]. In clinical practice, the use of antioxidants can improve the vision of AMD patients [15]. Antioxidants have been paid more and more attention and become the focus of research on the treatment of AMD.

Rb1, as an effective component with the largest content of ginsenosides [16], can improve the level of oxidative stress in aging mice [17]. Ginsenoside Rb1 can also inhibit the oxidative stress of RGC-5 cells of optic nerve [18]. Oxidative stress can induce the increase of ROS in RPE cells, causing damage to retinal function [19]. This study demonstrated that 4-HNE could induce oxidative damage in ARPE-19 cells; ginsenoside Rb1 can clear ROS induced by 4-HNE and promote the antioxidant damage of RPE cells, suggesting that Ginsenoside Rb1 has a certain protective effect on retinal oxidative damage.

Studies have shown that RPE cell function damage can cause apoptosis, and ginsenoside Rb1 has significant anti apoptosis activity [20,21]. The results of this study suggest that ginsenoside Rb1 can inhibit cell oxidative damage induced by 4-HNE, and further suggest that ginsenoside Rb1 can inhibit cell apoptosis caused by RPE cell function damage.

In conclusion, ginsenoside Rb1 has a protective effect on 4-HNE induced oxidative damage of ARPE-19 cells. Its mechanism may be related to the inhibition of oxidative stress reaction, which provides a new diagnosis and treatment strategy for clinical treatment of AMD and other retinal diseases.

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